Prognostic Score for Patients with Localized Renal Cell Carcinoma Treated by Nephrectomy

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Abstract

Objective: To evaluate the feasibility of using combined clinical and histomorphometric features to construct a prognostic score for the individual patient with localized renal cell carcinoma.

Patients and Methods: We studied 39 patients with pT1 and pT2 RCC who underwent radical nephrectomy between 1974 and 1983. Univariate and multivariate analyses were used to determine the association between various prognostic features and patient survival.

Results: The most important and independent predictors of survival were tumor angiogenesis ($P = 0.009$), nuclear DNA ploidy ($P = 0.0071$), mean nuclear area ($P = 0.013$), and mean elongation factor ($P = 0.0346$). Combination of these variables enabled prediction of outcome for the individual patient at a sensitivity and specificity of 78% and 89%, respectively.

Conclusion: Our results indicate that no single parameter can accurately predict the outcome for patients with localized RCC. Combination of neovascularity, DNA content and morphometric shape descriptors enabled a more precise stratification of the patients into different risk categories.

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A major problem in the management of patients with renal cell carcinoma is the prediction of the neoplasms' malignant potential, and in turn the patient's prognosis. This is due to the unpredictable clinical course in patients with RCC [1–4]. It has been estimated that following surgery, recurrence will occur in as many as one-third of patients with localized RCC, suggesting a biological heterogeneity of this tumor [5–8]. Various parameters have been used to assess the malignant potential of renal cell carcinoma, including clinical and pathological stage, histological grade, tumor size, nuclear morphology, immunohistochemistry, ferritin expression and angiogenesis. To date, the single best predictor of patient outcome is disease stage at the time of diagnosis [1–4]. However, no single studied variable is able to predict outcome on an individual basis [9–13].

The current study was undertaken to evaluate the feasibility of using combined predictive features to construct a prognostic score to accurately determine the prognosis of patients with localized renal cell carcinoma.

RCC = renal cell carcinoma

Patients and Methods

Patients

Data were obtained on 39 consecutive patients who underwent radical nephrectomy for pathologically confined RCC (pT1, pT2) between 1974 and 1983. The study group included 24 men (62%) and 15 women (38%) with a median age of 62 (range 35–84 years). The mean duration of follow-up was 7.6 years (range 5 months–16.7 years), and 9.7 years for surviving patients. All the patients were evaluated postoperatively at regular intervals – every 3 months during the first 2 years and every 6 months thereafter. Follow-up consisted of physical examination, chest X-ray, abdominal ultrasound or computed tomography, blood chemistry and bone or liver scan when clinically indicated. The original histological slides, stained with hematoxylin and eosin, were reviewed to confirm the diagnosis of RCC and to exclude the sarcomatoid variant. The patho-logical reports were reviewed for size, cell type, histological pattern (papillary versus non-papillary), tumor grade [14] and pathological stage [15].

Prognostic features

The following quantitative variables were studied for each case:

- Flow cytometric analysis of nuclear DNA content using the Hedley and Vindeløv techniques was performed. Tumors with cell samples that had histograms resembling those of normal renal parenchyma were categorized as normal or DNA diploid. A population of tumor cells that contained a significant increase in the G2 (4C) peak of the cell cycle was categorized as DNA tetraploid. If a sample had a third additional peak, different from the G0/G1 (2C) or G2 (4C) peaks, it was classified as aneuploid [16].
- Nuclear morphometry was performed using an interactive image-analysis system. The following shape descriptors were measured: a) mean nuclear area, with the cross-sectional area

measured in square microns; b) mean nuclear elongation factor, with the degree of ellipticity calculated as: (minimal diameter/maximal diameter) x 1000. Values of 1000 represented a circle while values less than 1000 represented an elliptical structure; c) mean nuclear regularity factor, which is the degree by which a nuclear contour has smooth or irregular borders, was calculated as: area/[(π/4) x major diameter x minor diameter] x 1000. Values of a 1000 represented smooth borders while values less than a 1000 represented irregular borders [5].

- Immunohistochemical staining for epithelial membrane antigen, cytokeratin, and vimentin expression [17].
- Tumor angiogenesis, determined as the number of microvessels in five high power (x400) microscopic fields that stained positive for factor VIII related antigen [18].

Statistical analysis

Univariate analysis for assessing the impact of various parameters on patient survival was performed using Cox univariate analysis. Cancer-specific survival curves were constructed according to the Kaplan-Meier product limit method. The log rank test was used for statistical comparison between survival curves. Cox's proportional hazard model was performed for multivariate analysis in order to detect significant and independent predictors of survival. Based on the regression coefficients (intercept and slope), a combined proportionality constant was calculated. The following formula was used:

$$e^{\alpha} + \beta_1 X_1 + \beta_2 X_2 + \quad + \beta_n X_n$$

where:

e = natural log

$\beta_1, \beta_2, \beta_n$ = the slopes of the independent variables

X_1, X_2, X_n = the individual values of each variable

α = intercept = $(- \beta_1 X_1 - \beta_2 X_2 - \quad - \beta_n X_n)$

The combined proportionality constant was then introduced in a receiver operating characteristic curve in order to find the best cutoff point (sensitivity and specificity) to predict survival beyond 60 months. Two-tailed *P* values of 0.05 or less were considered statistically significant.

Results

During the period of follow-up 18 patients (46%) died of renal cancer. Univariate association between the various prognostic variables and patient survival is presented in Table 1. The most significant prognosticator was nuclear DNA ploidy pattern ($P = 0.0005$), followed by mean nuclear elongation factor ($P = 0.0019$), mean nuclear area ($P = 0.004$), histological grade ($P = 0.0047$), and vimentin status ($P = 0.0076$). Kaplan-Meier survival curves constructed for the patients studied in the ploidy subgroups are shown in Figure 1. No patient with abnormal DNA content was alive 12 years postoperatively, while 65% of patients with DNA diploid tumors were ($P < 0.0005$) were. The median survival time for patients with non-diploid tumors was 36 months; the median survival time for those with normal DNA content has not been reached.

Multivariate interactions for patient survival were investigated by using a forward stepwise Cox regression model. After analyzing potential confounding variables and adjusting for multiple intervariable relationships, the most important prognostic variable was angiogenesis (microvessel density) ($P = 0.0009$). Other significant and independent predictors were DNA ploidy, MNA, and MNEF [Table 2]. Stratification of these predictors into prognostic categories revealed that the highest relative risk for death from RCC was related to nuclear size. Patients whose tumors had a MNA greater than $32\mu^2$ had a 12.1 increased chance of cancer death when compared to patients having tumor nuclei smaller than $32\mu^2$. ROC curves

Table 1. Relationship between various prognostic features and survival in patients with localized RCC

Variable	*P* value*
Ploidy	0.0005
MNEF	0.0019
MNA	0.0040
Grade	0.0047
Vimentin expression	0.0076
MNRF	0.0283
Angiogenesis	0.0445
EMA expression	0.0554
Cytokeratin expression	0.2067
Cell type	0.4435
Size	0.5724
Gender	0.6389
Architecture	0.8023
Age	0.9945

* Cox univariate analysis

AUC = area under the curve, EMA = epithelial membrane antigen, MNA = mean nuclear area, MNEF = mean nuclear elongation factor, ROC = receiver operating characteristic

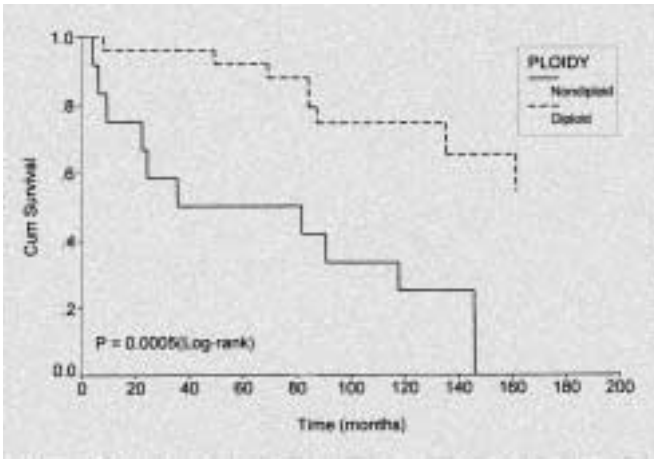

Figure 1. Postoperative probability of survival in patients with localized RCC for normal versus abnormal DNA ploidy pattern. ($P = 0.0005$, log-rank test)

Table 2. Multivariate association between prognostic features and survival in patients with localized RCC

Variable	β (slope)	*P* value*
Angiogenesis	0.1149	0.0009
Ploidy	1.5151	0.0071
MNA	0.0854	0.0130
MNEF	-0.0206	0.0346

* Cox multivariate analysis

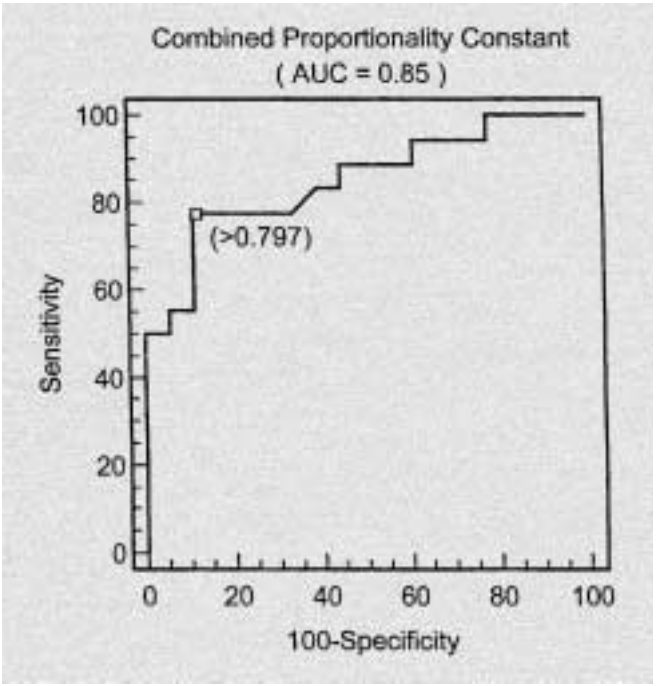

Figure 2. ROC curve for predicting survival in patients with localized RCC based on combined proportionality constant.

were constructed for each one of these variables. The area under the curve, which expresses the ability of any variable to correctly diagnose patient outcome, was estimated using Wilcoxon statistics. The best predictors were MNEF (AUC = 0.69, 95% CI 0.51–0.83) and microvessel count (AUC = 0.64, 95% CI 0.46–0.79). For prediction of outcome on an individual basis a combined proportionality constant was calculated. It included all relevant values (regression coefficient) of the independent variables according to the Cox model (angiogenesis, ploidy, MNA, and MNEF). Figure 2 depicts the ROC curve of the combined proportionality constant. At the threshold value of 0.797, the sensitivity:specificity ratio for survival over 5 years was 78% and 89%, respectively. The AUC is 0.85 with a 95% CI (0.69–0.94).

Discussion

A major problem encountered in the management of patients with localized RCC is the prediction of the neoplasms' malignant potential. In accordance with recent advances in immunological therapy, it is important to stratify the patients into risk categories [19,20]. Those patients who are more likely to develop metastases may benefit from early adjuvant therapy. The unpredictable course of patients with localized RCC may be a reflection of our inability to identify one sensitive and specific marker. Considering the extensive heterogeneity of RCC and the lack of a single predictive variable, we sought to develop an objective prognostic score based on combined measurable features.

We studied the impact of various clinical, histological, immunohistochemical, and morphometric parameters on the survival of patients with organ-confined RCC. The parameters with a significant predictive power [Table 2] were incorporated into a multivariate regression analysis according to the Cox model. The four significant and independent predictors were tumor angiogenesis, DNA ploidy pattern, nuclear size (MNA) and nuclear ellipticity expressed as MNEF [Table 2].

The prognostic importance of these variables has also been shown by others. Yoshino et al. [13] studied 45 cases of T1 and T2 renal cancer and found that the only significant predictor of outcome by multivariate analysis was tumor microvasculature. Ljungberg and co-workers [21] investigated the predictive value of nuclear DNA content using flow cytometry. They found that of the 59 evaluable tumors, cancer death occurred only in those displaying abnormal DNA ploidy patterns. The association of nuclear shape descriptor and survival in patients with renal cell carcinoma was reported by Gutierrez et al. and Murphy et al. [10,22].

The combination of these independent variables with the use of the proportionality constant enabled us to create a useful prognostic score with a sensitivity and specificity of 78% and 89%, respectively. The validity of the score is substantiated by the fact that all the tumors were confined to the kidney, which eliminates any potential confounding effect due to the extent of the disease (such as pathological stage). In addition, the long-term follow-up of our study group (mean 9.7 years for surviving patients) minimizes the possibility of late progression and death.

Attempts to combine predictors for determining prognosis were also performed by Pound and associates [23]. In their study, multivariate analysis of the four best individual shape descriptors correctly predicted prognosis in 23 of the 26 patients analyzed, with a sensitivity and specificity of 73% and 100%, respectively. A similar study, which analyzed the impact of clinical and karyometric features (n = 26) on survival of 121 patients with RCC, was conducted by Van der Poel et al. [24]. Using the Cox regression analysis of the best clinical and karyometric features, they were able to form prognostic groups in the entire population. The 3 year survival rate for patients with a good prognosis was 80%, compared to 21% for patients with a poorer prognosis.

Conclusion

Based on our results, it is likely that no single parameter can serve as a complete and accurate indicator of the outcome of patients with localized renal cell carcinoma. We found tumor neovascularity, DNA ploidy patterns, nuclear area and nuclear ellipticity to be significant prognosticators, and their combina-

tion resulted in an accurate stratification of patients into risk categories. Such information may be useful for creating follow-up protocols or considering adjuvant treatment for patients with early stage renal cancer.

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